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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Isolation, Identification and Determination of *Saccharomyces cerevisiae* Yeast Species from The Wines Made by Spontaneous Fermentation Using Papazkarası Grapes from Thrace Region

Trakya Yöresine Ait Papazkarası Üzümleri Kullanılarak, Spontan Fermantasyon ile Elde Edilen Şaraplardan *Saccharomyces cerevisiae* Türü Mayaların İzolasyonu, Tanımlanması ve Teknolojik Özelliklerinin Belirlenmesi

Çağrı ERSEÇ[1*], Ahmet Şükrü DEMİRCİ[2]

Abstract

Especially in large-scale commercial wine production, fermentation is usually carried out by using the species of *Saccharomyces cerevisiae* yeast cultures. Standard wine yeast starter cultures are brought from abroad and used in industrial winemaking in our country. Thus, in course of time, the wines of all producers began to show similar properties. Today, studies focus on terroir wines production with local characteristics by using the countries' own local wine yeasts as a starter. Within the scope of this study Papazkarası grapes were collected from 4 vineyards with different characteristics, such as viticulture practices, microclimatic conditions and locations in the borders of Edirne and Kırklareli. In addition, the grapes were also collected from the vineyard in Tekirdağ Viticulture Research Institute. Spontaneous fermentation was done by crushing the collected grapes in the laboratory. At the end of the fermentation, yeast isolations were made from the musts to YPD medium and a total of 66 isolates were obtained as a result of purification. Fermentation rate, H_2S production amount, growth at high temperature, growth at high sugar concentration, resistance to high ethanol, ability to grow at low pH values, resistance to SO_2, and volatile acid analysis were done to determine whether they meet the technological requirements for basic winemaking, together with two commercial wine yeasts. DNA sequencing analyzes were made by selecting 15 of the isolates that were found to have the necessary characteristics and it was determined that they belonged to the *Saccharomyces cerevisiae* species. DNA fingerprinting analysis was performed using delta 12-21 primers to determine the strain differences of the isolates determined to belong to the same species. By DNA fingerprint analysis, it was determined that 9 out of 15 isolates were different strains.

Keywords: Papazkarası, *S. cerevisiae*, Wine, DNA, Yeast

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Öz

Günümüzde, özellikle büyük çaplı ticari şarap üretiminde, fermantasyon genellikle *Saccharomyces cerevisiae* türü maya kültürleri kullanılarak gerçekleştirilmektedir. Standart şarap mayası starter kültürleri Uluslararası büyük maya üreticisi firmalardan alınarak ülkemizde, şarap yapımında kullanılmaktadır. Bu da, üreticilerin şaraplarının zamanla birbirine benzemesine yol açmaktadır. Günümüzde yapılan çalışmalar, ülkelerin kendi yerel şarap mayalarını starter olarak kullanarak yerel özellikler taşıyan teruar şaraplarının üretimine yoğunlaşmaktadır. Bu çalışma kapsamında, Edirne ve Kırklareli sınırlarında bulunan, yapılan bağcılık uygulamaları, mikroklimatik şartları, konumları gibi özellikleri farklılık taşıyan 4 bağdan ve Tekirdağ Bağcılık Araştırma Enstitüsündeki bir bağ parselinden olmak üzere toplam 5 farklı lokasyondan Papazkarası çeşidine ait üzümler önolojik olgunluk aşamasında toplanmıştır. Toplanan üzümler laboratuvara getirilerek ezilmiş ve sonrasında spontan fermantasyona bırakılmıştır. Fermantasyon sonunda fermante olmuş şıralardan, YPD besiyerlerine, ekim yapılmıştır. Besiyerlerinde gelişen kolonilerin saflaştırması işlemi sonucunda toplam 66 adet izolat elde edilmiştir. İzolatların, 2 adet ticari şarap mayası ile birlikte, temel şarap yapımı için gereken teknolojik özellikleri karşılayıp karşılamadıklarını belirlemek için fermantasyon hızı, H_2S üretim miktarı, yüksek sıcaklıkta gelişebilme, yüksek şeker konsantrasyonunda gelişebilme, yüksek etanole dayanıklılık, düşük pH değerlerinde gelişebilme, SO_2'ye dayanıklılık, uçar asit miktarı analizleri uygulanmıştır. Gerekli özelliklere sahip olanlardan 15 tanesi seçilerek DNA dizileme analizleri yapılmış ve *Saccharomyces cerevisiae* türüne ait oldukları tespit edilmiştir. Aynı türe ait oldukları belirlenen izolatların alt tür bazında birbirinden farklı olup olmadıklarını belirlemek üzere delta 12-21 primerleri kullanılarak DNA parmak izi analizi yapılmıştır. Bu izolatlara yapılan DNA parmak izi analizlerinde 9 adet farklı suş tespit edilmiştir.

Anahtar Kelimeler: Papazkarası, *S. cerevisiae*, Şarap, DNA, Maya, Karakterizasyon

1. Introduction

Wine is one of humanity's oldest beverages. The wine was originally formed by fermentation of damaged grapes in barrels (Chambers and Pretorius, 2010). Today, wine fermentation is carried out using yeasts such as *Saccharomyces cerevisiae* (Jolly et al., 2006). *S. cerevisiae*, which is also used in fields such as enzymes and bread production today (Özdinç and Velioğlu, 2022), is accepted as the main yeast species responsible for alcoholic fermentation due to its ability to grow in grape juice, which is characterized by high sugar and low nitrogen content. *S. cerevisiae* species produce high amounts of ethyl alcohol, inhibit the growth of other yeasts and become dominant during must fermentation (Cocolin et al., 2004).

The factors that determine the quality and character of the wine are the grapes used and the cultivation style, as well as the fermentation outputs of the yeast used. Standard yeast cultures produced worldwide are widely used in industrial winemaking. Thus, over time, the wines of all producers began to show similar characters. Today, studies focus on wine production with local characteristics by using the countries' own local wine yeast as a starter culture (Varela and Borneman, 2016). In addition, autochthonous yeasts show better adaptability to specific musts determined by terroir and grape variety (Bokulich et al., 2013).

Wine yeasts selected for use in wine production need to have certain technological properties (fermentation rate, H_2S production amount, growth at high temperature, growth at high sugar concentration, resistance to high ethanol, ability to grow at low pH values, resistance to SO_2, volatile acid etc.) that will make them suitable for industrial wine production, and the determination of these technological properties is very important for the efficiency of the fermentation process. Many of the technological features differ greatly within yeast strains. Therefore, the technological properties of all isolates must be determined. In addition, during the selection of yeasts, their genomic structures should be determined and defined as well as morphological differences (Lopes et al., 2006)

Turkey terroir is very suitable for the cultivation of different grape varieties (Tahmaz et al., 2022). Thrace region is one of the important wine grape growing regions in Turkey. Papazkarası grape variety is the indigenous and ancient grape variety of the Thrace region. Within the borders of Thrace, there are many Papazkarası vineyards with different characteristics. There is no study in the international literature on the isolation and identification of *Saccharomyces cerevisiae* yeasts suitable for winemaking from Papazkarası grapes in this region. Grapes harvested from Papazkarası vineyards with different characteristics in the Thrace region were used in this study since factors such as geography, altitude, climate, the presence of other grape varieties grown in the same vineyard, soil structure, and age of the vineyard are the most important factors on the yeast population and variety on the grape.

The aim of the study was to isolate, identify and determine the technological properties of Saccharomyces cerevisiae yeast strains from microvinification experiments with Papazkarası varieties collected from vineyards in the Thrace region. Thus, it will be contributed to the process of collecting local yeasts from our country's own genetic resources, identifying suitable and resistant species, and selecting yeasts strains suitable for winemaking, in order to produce wines with unique flavors with high international competitiveness. In addition, since the isolated and identified wine yeast candidates will be stocked, a contribution will be made to the gene bank of our country and a resource will be created for future studies.

2. Materials and Methods

2.1. Region

Grapes to be used in the study were harvested at maturity stages from 5 vineyards located within the borders of Edirne, Kırklareli and Tekirdağ (*Figure 1*).

- Kirklareli, Üsküp : 41°45’10”N 27°23’30”E, Altitude: 351m
- Kirklareli, Hamitabat : 41°32’00”N 27°17’41”E, Altitude: 140m
- Edirne, Kircasalih : 41°22’52”N 26°48’6”E, Altitude: 87m
- Edirne, Yeniköy : 41°20’18”N 26°45’17”E, Altitude: 78m
- Tekirdağ Viticulture Research Institute : 40°58’30”N 27°28’03”E, Altitude: 50m

While determining the technological properties of isolated yeasts, 2 commercial wine yeasts (RC212, ICV Opale)

were used. *Candida krusei* strain, which is known to be a strong producer, was used to determine the level of H_2S production.

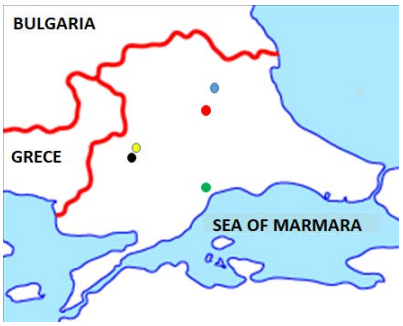

Figure 1. Regions where Papazkarası grapes are harvested

2.2. Spontaneous fermentation process

25 mg l^{-1} sulfur dioxide (SO_2) has been added to avoid any biological risk but to ensure the greatest possible yeast population in the must (Parish and Carroll, 1987). Musts were left to ferment at a constant temperature of 17°C. Until the end of the fermentation, the weight loss in the bottles was measured every day and it was predicted that the fermentations were finished when the weight loss stopped. In all prepared trials, fermentations were completed between 15-25 days.

2.3. Isolation

At the end of the fermentation, samples were taken from the musts and inoculated on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, 1.5% agar) containing 0.015 mg l^{-1} amoxicillin at concentrations of 10^{-2} to 10^{-6} and Incubated at 28°C for 4 days (Çavdaroğlu, 2017). At the end of the incubation, the colonies were randomly selected according to their morphological characteristics and purified by cultivating on another YPD agar with the streaking method. The purification process was repeated 6 times (Valero et al., 2007).

2.4. Identifying technological features

H_2S Production: The rotten egg smell created by hydrogen sulfide in wines is an undesirable situation. Therefore, yeasts to be used as wine starter cultures are required to produce low levels of H_2S. Isolated yeast strains were cultivated on BIGGY (bismuth sulphite glucose glycine yeast) agar and incubated at 30°C for 5 days, and hydrogen sulfide production was estimated by looking at the colour formation on the medium. The degree of browning of yeast lines; 1: white, 2: cream, 3: light brown, 4: brown, 5: dark brown and 6: black (Cordente et al., 2009).

Fermentation Rate: It is desired that wine yeasts become dominant in the must and suppress other microorganisms in the first stages of fermentation. In this study, the growth rate of the isolates was determined by measuring the weight loss that occurred in the media between the 24^{th} and 72^{nd} hours of fermentation. Inoculations were made by putting 20 mL of YPD medium with a pH of 3.5, containing 20% glucose in 50 mL sterile tubes, and the tubes were left to incubate at 25°C. The weight loss due to CO_2 gas output between the 24^{th} and 72^{nd} hours of fermentation was calculated in "g CO_2 $(L\ h)^{-1}$" (Pérez-Coello et al., 1999)

High Temperature Tolerance: Yeast strains were inoculated in YPD broth (10 mL), incubated at 37 and 42°C for 5 days, and their growth was examined (Nikolaou et al., 2006).

High Sugar Tolerance: Yeast cultures were inoculated into 10 mL YPD Broth medium adjusted to 30°Bx, and incubated at 30°C for 3 days. At the end of the period, the growth of gas-forming isolates in two-thirds of the Durham tube was considered positive (Iranzo et al., 1998).

High Ethanol Tolerance: After counting with a Thoma slide (~3 µL 10^6 cfu mL-1), yeast isolates are inoculated into a 10 mL YPD medium, containing 100-130-150-170 mL L^{-1} ethanol and incubated at 30°C for 72 hours. Their growth at the end of this period was examined (Guimarães et al., 2006).

Low pH Tolerance: YPD media with pH values adjusted to 3.0 and 4.0 with HCL and NaOH were put in 15 mL tubes as 10 mL. Isolates are inoculated (10 µL) and incubated at 28°C for 7 days. At the end of this period, the gas accumulation in the tubes was examined. Unadjusted YPD medium with a pH of 6.67 was used as a control in the

experiment. (Charoenchai et al., 1998).

Sulfur Tolerance: The growth characteristics of the isolates on YPD media supplemented with SO_2 at concentrations of 0, 50, 100, 150, and 200 mg L^{-1} were investigated. Activated yeast cultures were inoculated into the medium at a level of approximately 10^6 cells mL^{-1} and incubated at 28°C for 72 hours. (Guimãraes et al., 2006).

Volatile Acid Production: YPD medium containing 20% glucose was used to detect for volatile acid production. The steam distillation method was used to determine the amount of volatile acid formed by yeast in the medium. Sixty mL of liquid distilled by steam distillation system was heated to boiling point. Then, it was titrated with 0.1 N NaOH solution under phenolphthalein indicator until colour change was observed. (Ough and Amerine, 1988).

2.5. Molecular characterization

DNA sequencing analyses: Among 66 isolated yeast strains, sequencing and DNA fingerprinting analyzes were performed for 15 yeast strains that showed proficiency in technological tests.

After using the lyticase enzyme to break down the yeast cell walls, the steps specified in the "Wizard Genomic DNA purification Kit" product of Promega company were followed. The purity of the isolated DNA was determined by nanodrop.

In the studies, ITS1 (5´TCC GTA GGT GAA CCT TGC GG 3´) was used as the forward primer and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´) was used as the reverse primer. In the study, 17.5 µL nuclease-free sterile water, 2.5 µL buffer (does not contain $MgCl_2$), 0.5 µL (deoxynucleotide triphosphate) dNTPmix, 0.5 µL ITS1 forward and 0.5 µL ITS4 reverse primers, 2 µL $MgCl_2$, 0.5 µL Taq DNA polymerase enzyme and 50 ng DNA were added in a total volume of 50 µL in 500 µL PCR tubes. PCR reaction parameters were programmed as 15 min initial denaturation at 95°C, 60 s denaturation at 94°C, 2 min annealing at 55°C, and 2 min extension at 72°C, and this process was repeated 35 times. PCR was completed with a 10 min final extension at 72°C (Blaiotta et al., 2002). Sequence analysis of DNA samples visualized on agarose gel was performed at Namık Kemal University Scientific and Technological Research Application and Research Center. The nucleotide sequences of yeast samples were compared with known sequences in NCBI GenBank using the BLAST program.

DNA fingerprinting analysis: To see the differences in yeast samples based on strain, DNA fingerprinting analyzes were performed using delta12 (5´TCAACAATGGAATCCCAAC´3) and delta21 (5´CATCTTAACACCGTATATGA´3) primers. The PCR reaction contains 25 µL volume of 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1 µM forward primer (delta 12), 1 µM, reverse primer (delta 21), 1.25 Units of Go-taq flexi DNA polymerase (Promega), 50 ng DNA. PCR conditions were pre-denaturation at 95°C for 4 min, then 30 cycles of 30 s at 95°C, annealing at 46°C for 30 s, polymerization at 72°C for 90 s, and finally a final extension at 72°C for 7 min. Post-PCR samples were run on a 1.4% agarose gel in 1xTBE buffer at 100 V for 2 hours. In the presence of ethidium bromide, they were viewed under UV light and their photographs were recorded (Bilgin, 2015).

3. Results and Discussion

With the weightings made during the spontaneous fermentation process, data such as the start times of fermentations, their speeds in certain periods, and the finishing stages were obtained (*Figure 2*).

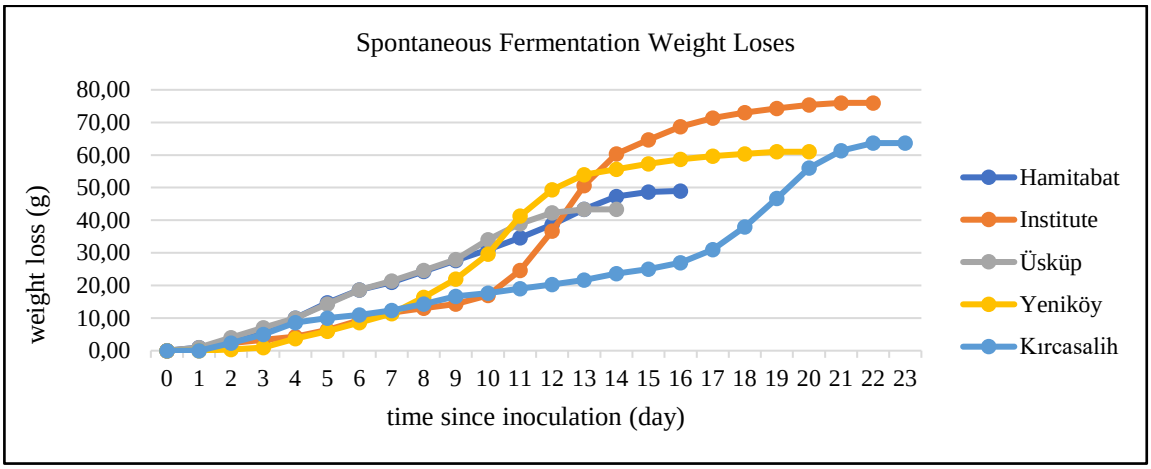

Figure 2. Weight reductions of grape must collected from different vineyards during spontaneous fermentation

It was observed that fermentations in all samples except Yeniköy started in 2-3 days, and in Yeniköy samples, fermentations started on the 4th day. This time may vary depending on the density of the microorganism flora in the samples and/or the capabilities of the microorganisms initiating fermentation. In addition, the completion times and rates of fermentations differed from each other, which is due to the differences in the initial sugar, water-soluble dry matter and microbial flora on the peel (Lu et al., 2020).

3.1. Isolation and sampling

When the weight reductions were fixed, samples were taken from each of the fermented musts (Guimarães et al. 2006). A total of 66 isolates were purified. The isolates were named as the initial letter of the region they were taken from and the isolate number (H9: 9. Isolate obtained from the vineyard in Hamitabat region).

3.2. Analyzes to Determine the Technological Characteristics of Yeasts

H_2S Production: Since the rotten egg odor created by hydrogen sulfide in wines is undesirable, yeasts to be used as wine starters are required to produce low levels of H_2S (*Figure 3*).

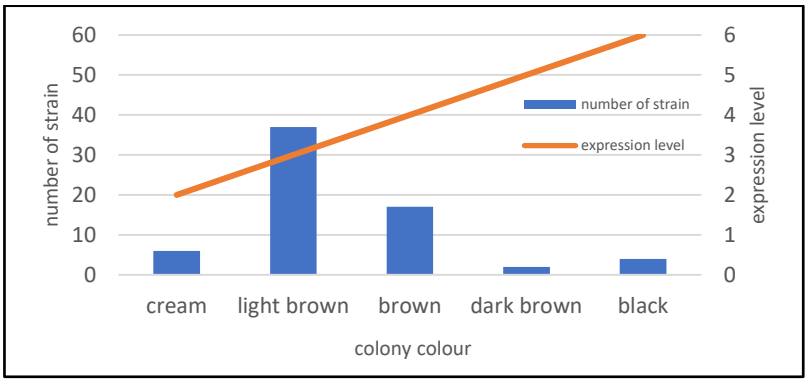

Figure 3. H_2S production levels according to colony colours formed by isolates on BIGGY agar

In the hydrogen sulfide production analysis, 6 strains formed cream-coloured (2) colonies on BIGGY agar, while 37 light brown (3), 17 brown (4), 2 dark brown (5) and 4 black (6) colours were formed. Commercial yeast strains taken as references formed a brown colour, indicating that they are intermediate hydrogen sulfide producers. 60 of the isolates that formed brown and lighter coloured colonies were accepted as potential wine starters. 6 isolates (Ü1, Ü6, Ü8, Ü9, Ü16 and Ü19) forming dark brown and darker colonies were evaluated in the category of yeast that does not have wine-making properties due to their high hydrogen sulfide production. It has been observed that 5 out of 6 isolates (Y7, Y8, Y9, H6, E1), which give cream colour, have been successful in other technological analyzes as well, so they have a high potential to be used in the production of wines that are required to have very low H_2S levels from yeast or to be made from damaged grapes.

In similar studies, it is seen that the majority of the isolated *S. cerevisiae* strains give light brown and brown colony colour (Budroni et al., 2006; Lopes et al., 2007; Bağder, 2008; Çelik et al., 2017).

Fermentation Rate: In the experiments, all yeasts, except for 6 isolates (Ü1, Ü6, Ü8, Ü9, Ü16, Ü19), which were found to have no ability to ferment the must, caused a weight reduction greater than 0.25 g CO_2 $(L\ h)^{-1}$ between 24-72. hours. (Iranzo et al., 1998).

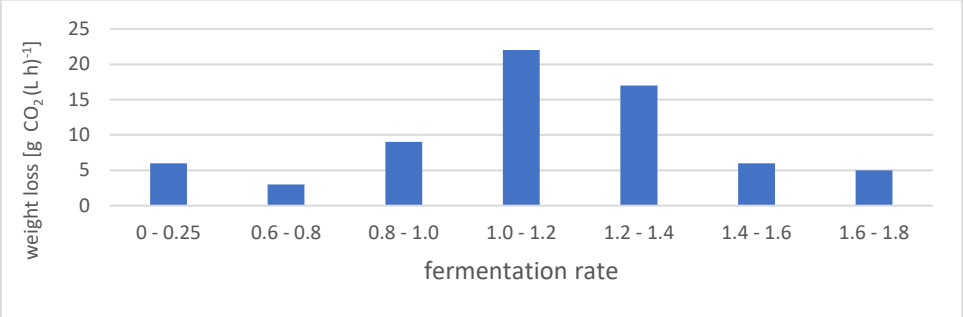

Figure 4. Weight reductions in the media between the 24th and 72nd hours of inoculation

The weight reductions of the other yeasts in the study were found to be in the range of 0.739 - 1.698 g CO_2 $(l\ h)^{-1}$. From this point of view, in terms of fermentation rate, it is seen that all other isolates can be used as starter cultures in wine. It was observed that 36 of the isolates had higher fermentation rates than the control group, commercial red wine yeast (RC212), and 30 of them had higher fermentation rates than the control group, commercial white wine yeast (ICV Opale). It was determined that the isolates leading in weight reductions at the end of fermentation were also prominent in weight reductions between 24-72 hours (*Figure 4*). Yeasts with a high fermentation rate are those isolated from grapes collected in Yeniköy and Hamitabat, proportionally. According to other studies on this subject, it has been observed that more than half of the isolates are dominant yeasts with a high fermentation rate that can ferment the must very strongly (Pérez-Coello et al., 1999; Orlić et al., 2005; Budroni et al., 2006; Bagder, 2008).

Table 1. Technological tests of isolates

Other technological tests of isolates	Number of tolerant isolates	Number of intolerant isolates
High temperature tolerance (37°C)	66	-
High temperature tolerance (42°C)	56	10
Growth at 30°Bx	66	-
Ethanol tolerance (10%)	64	2
Ethanol tolerance (13%)	57	9
Ethanol tolerance (15%)	53	13
Ethanol tolerance (17%)	10	56
pH tolerance (3.0)	66	-
pH tolerance (4.0)	66	-
SO_2 tolerance (50 ppm)	66	-
SO_2 tolerance (100 ppm)	65	1
SO_2 tolerance (150 ppm)	64	2
SO_2 tolerance (200 ppm)	64	2

High temperature tolerance: It has been observed that all isolated strains can grow at 37°C. Only 10 of 66 isolates (K6, K8, Ü1, Ü2, Ü6, Ü9, Ü17, H8, H10 and H15) failed to grow at 42°C (*Table 1*). This rate is seen as a high rate compared to the development of isolates in similar studies at these temperatures (Bağder, 2008; Çavdaroğlu, 2017; Antia et al., 2018). With this analysis, the temperature resistance of the majority of isolated yeasts was found to be suitable for winemaking. Yeasts that grow well at high temperatures have the potential to be used to prevent stuck fermentation in situations where there is no cooling system and extreme temperature rises can be observed during fermentation. 5 of the 10 isolates that could not grow at 42°C were yeasts isolated from grapes in the Üsküp region. Since the sugar content of the grapes in this vineyard was relatively low (14°Bx) at the harvest stage, the alcohol content at the end of the fermentation was also relatively low. Therefore, it was considered that some of the yeasts isolated from the grapes collected from the Üsküp region may be *non-Saccharomyces* yeasts, and it is estimated that the isolates that did not grow at 42°C are *non-Saccharomyces* yeasts (Ali and Khan, 2014). Control yeasts showed growth at both temperatures.

High Sugar Concentration Tolerance: It was observed that all isolates in the study were able to grow at 30°Bx (*Table 1*). From this point of view, it's seen that the isolates can be used as starters resistant to osmotic pressure in the production of wines to be obtained from musts with high sugar concentrations obtained from grapes harvested late, grown in hot climates or obtained from grapes with botrytis. In other studies, it is seen that *S. cerevisiae* is generally resistant to this sugar concentration (Kuchen et al., 2019).

Ethanol Tolerance: Ü16 and Ü19 isolates were not evaluated as wine starters since they could not grow in 10% alcohol and K6, Ü9, H8, H9, H11, H12 and H13 isolates could not grow in 13% alcohol. On the other hand, K9, Ü2, Ü6 and Ü17 isolates were able to grow at 10% and 13% concentrations, but not at 15% concentration (*Table 1*). The use of these yeast strains in the production of high-alcohol wines can lead to potential problems. However, it is thought to have the potential to be used in the production of white wine or lower alcohol wines. The possibilities of using them in such areas have the quality to be researched in further studies. 53 isolates were able to grow at 15% ethanol concentration. The fact that they can develop at this concentration is a sufficient property for wine making and shows that they can be used as starters (Gerőcs et al., 2020). In addition, K3, K5, K7, K8, K10, K11, K12, K13, Ü3 and Ü18 isolates grew even at 17% ethanol concentrations. Tests for resistance to ethanol concentrations of 15% and above are

mostly used for selecting starter cultures to be used in bioethanol or biomass production. However, considering that ethanol is a stress factor on yeast, it can be predicted that the increase in the resistance of yeast to be used as a starter culture to ethanol will reduce the possibility of producing secondary metabolites that are produced under stress and can negatively affect the quality. For this reason, it was decided to select the isolates, which are thought to be starter cultures, from among the strains that can grow at 17% ethanol concentration. In percentage terms, the results are very similar to the other studies (Antia et al., 2018; Gerőcs et al., 2020).

pH Tolerance: In the analysis, it was revealed that all isolates and control strains were resistant to pH 3.0 and 4.0 stress factors and could grow at these pHs (*Table 1*). No difference was observed between the isolates and it was observed that all durham tubes were completely filled with gas. With this result, it was observed that the isolates were capable of initiating fermentation at low must pH's due to factors such as grape variety, grape maturity, and peel - grain ratio.

According to the study by Lu et al. (2016), it is seen that the growth of the isolated yeast species in our study is good in terms of resistance to must pH, which is one of the strong stress factors.

Sulfur Dioxide Tolerance: All isolates except E9 and E10 showed growth at SO_2 concentrations of 0-200 ppm (*Table 1*). While 200 ppm SO_2 concentration is determined as the upper limit for white wines with a residual sugar content of less than 4 g L^{-1} according to the International Organization of Vine and Wine (OIV) standards, this rate is 150 ppm for red wines with the same characteristic (OIV, 2021). It is thought that the isolates in the study can be used in cases where the high sulfur application may be required (low acid must, white grape must, processing of bruised grapes into wine etc.). The results are compatible with similar studies (Nurgel, 2000; Nikolaou et al., 2006; Bağder, 2008; Çavdaroğlu, 2017; Çelik et al., 2017)

Volatile Acid production: The volatile acid production potentials of 15 yeasts selected from among the isolates whose technological properties were determined, suitable for winemaking were investigated. According to OIV standards, it was observed that they formed less than 1.2 g L^{-1} of acetic acid (*Table 2*), which is the highest volatile acid concentration that wines can have. The results are similar to the other studies (Tristezza et al., 2014; Çelik et al., 2017; Furdíková et al., 2017). The obtained values were found to be average when compared with the other studies. In the Tukey multiple comparison test, it was observed that the volatile acid production degrees of the isolates were not statistically different from each other.

Table 2. Amount of volatile acid produced by isolates

Isolate no	Volatile acid (g L^{-1})	Isolate no	Volatile acid (g L^{-1})
K1	0.336 ± 0.021	Ü18	0.348 ± 0.094
K10	0.420 ± 0.098	H4	0.408 ± 0.138
K11	0.332 ± 0.132	H5	0.268 ± 0.045
Y4	0.328 ± 0.028	H6	0.276 ± 0.012
Y7	0.304 ± 0.037	E2	0.328 ± 0.037
Y9	0.352 ± 0.091	E4	0.292 ± 0.048
Ü3	0.348 ± 0.072	E7	0.256 ± 0.007
Ü14	0.328 ± 0.014	RC212	0.280 ± 0.018

3.3. Characterization

ITS gene regions were read in both directions and nucleotide sequences were compared with each other. The regions showing differences were evaluated manually on DNA sequencing chromatograms and prepared for BLAST analysis. Sequence analysis results were compared with the existing sequences in GenBank with the BLAST program and molecular diagnoses of yeast samples were made. As a result of the sequencing analysis, it was determined that all of the 15 selected isolates belong to the species *Saccharomyces cerevisiae*.

In the results obtained by sequencing analysis, after it was determined that all 15 isolates belonged to the *S. cerevisiae* species, it was decided to apply DNA fingerprinting analysis to all selected isolates in order to evaluate the differences on the basis of strains.

DNA samples amplified in PCR with Delta 12-21 primers were run in 1xTBE gel containing 1.4 agarose at 100V

for 2 hours and their images were recorded (*Figure 5*). The gel image was found to be similar to the images taken in studies in this area (Legras and Karst, 2003; Bilgin, 2015; Garofalo et al., 2016).

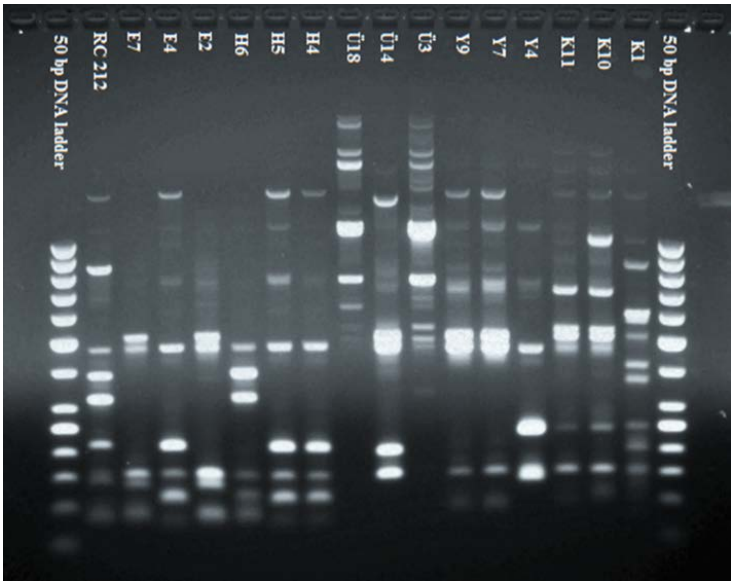

Figure 5. Gel electrophoresis image of DNA amplified with delta 12 - delta 21 primers

When the image recorded after electrophoresis was examined, it was seen that K10 - K11, Y7 - Y9, Ü3 - Ü18, H4 - H5 and E2 - E7 isolates were identical to each other. Interestingly, it was observed that the E4 isolate from a different vineyard was the same strain as the H4 and H5 isolates. It was observed that none of the yeasts identified in this study were the same strain as the commercial yeast (RC212). As a result of DNA fingerprinting using Delta 12-21 primers, it was seen that 9 of the selected strains were different *S. cerevisiae* strains.

4. Conclusions

Obtained isolates are stored in glycerol stocks at -80°C in Tekirdağ Viticulture Research Institute. Storage of isolates is important in terms of providing resources for future studies or applications. Future studies may be needed to determine whether these yeasts, whose basic winemaking competencies have been demonstrated, can produce quality wine.

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